



Conference Abstract

Surveillance of non-indigenous invertebrate species through DNA metabarcoding in recreational marinas in the North and Center of Portugal

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Abstract

DNA metabarcoding has been widely used in biodiversity assessments as a complement to traditional morphology-based techniques. This technique is useful for the early detection and surveillance of non-indigenous species (NIS) in aquatic ecosystems. When introduced to new locations, NIS can establish, and become invasive, which may cause severe alterations to native ecosystems and biodiversity. As most introductions in coastal ecosystems occur by transport in ships (through ballast waters discharges and biofouling), ports and marinas are priority hubs for the early detection of NIS. The aim of this study was to survey marine invertebrate NIS in four marinas in the north and center of Portugal using (e)DNA metabarcoding and different sampling methodologies.

We sampled four marinas in three locations in Portugal spanning a distance of ca. 150 km: one in Viana do Castelo (VC), one in Porto (L), inside the port of Leixões, and two in Aveiro (Av1: Costa Nova and Av2: Oudinot). Viana do Castelo, Porto, and Oudinot marinas are more sheltered, located near the coast, with a lower current flow, while Costa Nova marina is located inside a channel, more exposed to the passage of ships and to a higher current flow. Samples were taken of the organisms colonizing the marinas' hard substrates (e.g.,

pontoons, buoys, ropes), as well as from water, for environmental DNA (eDNA) analysis, and zooplankton, collected using a 55- μ m mesh net. We used two molecular markers: the mitochondrial cytochrome c oxidase gene (COI) and the small subunit ribosomal RNA gene (18S) to produce amplicon libraries for each sample, which were high throughput sequenced in an Illumina MiSeq platform. mBRAVE and SILVAngs were then used to process the sequence data and to generate species-level assignments for COI and 18S data, respectively.

A total of 23 NIS was detected in this study: 13 using each molecular marker. Only 3 NIS were detected using both markers. The highest number of NIS was detected in the Av1 marina with COI (11), whereas in the other marinas this number ranged from 5-9 NIS depending on the molecular marker. Six NIS were common to all the marinas, amongst which *Amphibalanus amphitrite* and *Amphibalanus eburneus*, two invasive barnacle species and *Eriocheir sinensis*, the Chinese mitten crab, a very well-known species with invasive behavior that causes severe ecological and economic damages. Fifty percent of NIS belonged to the phyla Arthropoda: Crustacea and Chordata: Ascidiacea. The sampling method that registered the highest number of NIS (9) was the eDNA from water in Av1, using COI. Overall, 18S detected approximately 1,6 times higher numbers of OTUs (237-267), phyla (16-21) and species (179-210) than COI, and only 5-6% of species were detected using both markers. Regarding the type of sampling method, the zooplankton samples retrieved the highest number of phyla (20 in VC; COI: 12 and 18S: 20) and more species (40 to 155; COI: 90 and 18S: 155), namely from Mollusca and Annelida. Between the three methods, there were only 21 (COI) to 28% (18S) of shared species. Crustacea dominated most samples collected from hard substrates. As for marinas, VC had the highest number of recovered OTUs (267), phyla (21) and species (210), when analyzed with the 18S marker, and the lowest number of OTUs (94) and species (86) was recovered in Av1 with COI. Coincidentally, this was also the marina with the highest number of NIS probably due to its more exposed location.

These results show the efficiency of NIS detection using (e)DNA metabarcoding but reveal the need to employ different molecular markers and sampling methodologies to guarantee a more comprehensive surveillance and detection of NIS in these environments.

Keywords

Coastal ecosystems; non-indigenous species (NIS); biosurveillance; (e)DNA metabarcoding; marine invertebrates

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